

transfections). These results were also validated using primary human OA chondrocytes.

**Results:** Sequencing of a 10kb fragment upstream of human Pitx1 promoter allowed us to identify a SNP in a potential E2F response element located in -3900/-3800 region of Pitx1 gene promoter. However, no association was found after validation of this SNP in a larger OA patient cohort (n=150). Nevertheless, using a 30bp probe, corresponding to the response element, and a DNA pull-down approach followed by mass spectrometry analysis (MS/MS), we discovered Prohibitin (PHB1) as the protein that interact with this response element. We confirmed this result with DNA pull-down followed by a Western blot against PHB1 and by chromatin immunoprecipitation (ChIP) assay. We have also found that in human OA chondrocytes, PHB1 is mostly accumulated in nucleus whereas in control subject it is mostly localized in cytoplasm. Overexpression of PHB1 in chondrocytes clearly demonstrated that PHB1 represses Pitx1 at mRNA and protein level. We also confirmed this repression using a gene reporter approach and point out that PHB1 use the -3900/-3800 response element discovered serendipitously. It has been shown that PHB1 represses the genes that are normally regulated by E2Fs transcription factor. Using a Luciferase reporter gene assay, we demonstrated that E2F1 is a transcriptional activator of Pitx1 gene and PHB1 over-expression block E2F1 effect on Pitx1 gene expression.

**Conclusions:** In healthy subjects, Pitx1 is normally regulated by E2F1, but in OA condition, accumulation of PHB1 in chondrocyte nucleus leads to Pitx1 gene repression. Further work will be undertaken to determine the mechanism that lead to nuclear PHB1 accumulation in OA chondrocytes.

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### THE PROTECTIVE ROLE OF THE PERICELLULAR MATRIX IN CHONDROCYTE APOPTOSIS

H.C. Peters, Z. Zhang  
Saint Louis Univ., St. Louis, MO

**Purpose:** In articular cartilage, pericellular matrix (PCM) plays a crucial role in homeostasis of chondrocytes. Apoptosis of chondrocytes contributes to common arthropathy such as osteoarthritis. This study attempted to quantify the protective role of the PCM in chondrocyte apoptosis using chondrons, which are a cartilage functional unit including a chondrocyte and its pericellular matrix.

**Methods:** Chondrocytes and chondrons were enzymatically isolated from human articular cartilage and exposed to moniodoacetate (MIA) and staurosporine for apoptosis induction. To identify chondrons, a basic component of the PCM, type VI collagen, was fluorescently labeled. Apoptosis of chondrocytes and chondrons was examined using flow cytometry and microscopy. Apoptosis and cell death were separately calculated in chondrons, the type VI collagen positive cell population, and chondrocytes.

**Results:** Chondrocytes treated with MIA incurred a 26.97% increase in overall cell death compared to only 11.79% in chondrons. Chondrocytes treated with MIA underwent a 9.06% increase in apoptotic cells compared to only 1.64% in chondrons. Similarly, chondrocytes treated with staurosporine underwent a 13.64% increase in apoptotic cells while it was a 3.22% increase in chondrons. TUNEL staining revealed that apoptotic chondrocytes often were the core of cell aggregates, while apoptotic chondrons did not attract cell aggregation. Caspase3 was expressed in both stressed chondrocytes and chondrons.

**Conclusions:** The PCM, a native microenvironment of chondrocytes, plays a protective role in chondrocyte survival. Retention of the PCM with chondrocytes is critically important in situations such as cartilage repair and tissue engineering, which depend on the survival of chondrocytes.

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### DIETARY HISTONE DEACETYLASE INHIBITORS AS CHONDROPROTECTIVE AGENTS

R.K. Davidson, K.L. Culley, Y. Bao, I.M. Clark  
Univ. of East Anglia, Norwich, United Kingdom

**Purpose:** Organosulfur compounds such as sulforaphane and allicin occur naturally in plants and are part of the normal diet. Sulforaphane can inhibit tumourigenesis in animal models, has anti-inflammatory properties and of particular interest, is reported to inhibit histone deacetylase activity (Myzack et al; 2004). Allicin, the pungent compound found in garlic, and related allyl sulfur compounds have been shown to inhibit the proliferation of several human tumour cell lines but not normal cells. These compounds have been shown to modify DNA methylation and acetylation (Lea et al; 2002). Histone deacetylase inhibitors are potential chondroprotective agents (Young et al; 2005, Xu et al; 2006). The aims of this project are to test the relative efficacy of dietary organosulfur compounds as HDAC inhibitors in human chondrocytes and to test the ability of such compounds to inhibit cartilage resorption in a bovine nasal cartilage explant model.

**Methods:** Sulforaphane (SFN) and diallyl disulfide (DADS) were tested for their relative efficacy in modulating cytokine-induced metalloproteinase expression and histone acetylation in chondrocytes using quantitative RT-PCR and Western blotting. Furthermore, these compounds were tested for their ability to prevent cartilage destruction in the bovine nasal cartilage assay. Lactate dehydrogenase assays were used to test for toxicity.

**Results:** Both SFN (5-10 $\mu$ M) and DADS (4-32 $\mu$ M) significantly attenuated IL-1 $\beta$ /oncostatin M-induced MMP1, MMP3, MMP13, and ADAMTS4 expression in SW1353 chondrosarcoma cells. MMP2 and MMP28 were not affected. Basal ADAMTS5 expression was repressed by SFN at 5-15 $\mu$ M. Global and histone H3 acetylation was not affected by SFN or DADS treatment in the SW1353 cell line. Cytokine-induced cartilage destruction was abrogated in a dose dependent manner by SFN (5-30 $\mu$ M) and DADS (8-32 $\mu$ M), measured by collagen and glycosaminoglycan release.

**Conclusions:** Type II collagen and aggrecan are major structural components of cartilage. MMP-1 and MMP-13 are key collagenolytic metalloproteinases in arthritic disease since they can degrade collagen type II. ADAMTS-4 and ADAMTS-5 have aggrecanase activity. SFN and DADS can attenuate the induction of these genes in a dose dependent manner but do not appear to function through the inhibition of histone deacetylases. SFN and DADS are potential chondroprotective agents.

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### PHOSPHATE AND CALCIUM INFLUX ARE REQUIRED FOR TGF $\beta$ -MEDIATED STIMULATION OF ANK (*progressive ankylosis*) EXPRESSION AND PPI TRANSPORT FUNCTION DURING CHONDROGENESIS

P. Oca, R. Zaka, A.S. Dion, C.J. Williams  
Thomas Jefferson Univ., Philadelphia, PA

**Purpose:** Genetic studies of familial chondrocalcinosis and cranial metaphyseal dysplasia have identified ANK, a multipass transmembrane protein, as a key player in biomineralization. The expression of ANK is stimulated by treatment of a variety of cell types with growth factors, including TGF $\beta$ . The purpose of this study was to determine whether TGF $\beta$  stimulation of ANK expression and function during chondrogenesis was dependent upon the influx of calcium and phosphate into the cells.

**Methods:** ATDC5 cells were differentiated from immaturity through hypertrophy with and without the addition of TGF $\beta$ 1. Inhibitors of TGF $\beta$  signaling were used to identify the signaling pathway em-